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(54) Title: HEAT TREATING LIPOSOMES

(57) Abstract

A new method is disclosed for sterilizing or heat treating liposomes. The liposomes may be empty or contain a bioactive agent; such liposomes may be administered in any pharmaceutically acceptable fashion such as parenterally or topically. The method involves subjecting liposomes to heating at a sufficient temperature and for a sufficient time to achieve sterilization.

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HEAT TREATING LIPOSOMES

Field of the Invention

This invention is concerned with a process for heating liposomes particularly for sterilization. The liposomes may contain a bioactive agent. More particularly, the present invention is concerned with sterilizing liposomes by autoclaving.

Background of the Invention

The concept of developing drug carriers for greater specificity and duration of drug action has received increasing attention in recent years. Liposomes have received a great deal of attention in this regard. Liposomes offer great potential in drug administration including parenteral, topical or extracorporeal use because of their versatility in terms of size and electrical charge. They are further able to contain both hydrophilic and lipophilic drugs. Their relative nontoxicity in comparison with other carrier systems is also advantageous.

Heating of liposome preparations is useful to cause physical and chemical changes in components of the liposome preparation, but such heating does not destroy the liposomes. In addition, pharmaceutical parenteral products in most instances must be sterile. The objective of sterilization is to remove or destroy all microorganisms or other heat labile contaminants in or on a preparation and to assure in this way that the preparation is free of infectious or other hazards when used with a patient. Since the

variety and amounts of sterile materials required for health care have increased in significant proportions in recent years, sterilization technology has become increasingly important. An essential element, therefore, in the preparation of modern-day pharmaceuticals is sterilization as well as verification that sterilization has been achieved.

Heating pharmaceutical preparations by any method such as boiling, microwave radiation, hot air and steam sterilization (autoclaving) are well known.

Even though liposomes have received a considerable amount of attention in recent years, very little information is available on methods for their sterilization. Liposome Technology Vol. 1, Chapter 10: "The Preparation of Sterile Drug-Containing Liposomes", Jurgen Freise; CRC Press, Boca Raton, Florida (1984).

Lipid emulsions, which are basically different from liposomes because of their bilayer structure, are currently manufactured by processes that involve a terminal autoclaving step, that is, sterilization of the lipid emulsions after preparation. However, it is widely written that liposomes are "fragile" and cannot withstand a sterilization cycle. It has been thought that the surfactant coating on lipid droplets tends to be "peeled" off when the temperature is raised too high resulting in demulsification.

Crommelin, D.A.J. et al, "Pharmaceutical Aspects of Liposomes - Preparation, characterization, and stability", Paperback-APV 17:80-191 (1987) suggests the terminal sterilization of a liposome product by filtration through a membrane with pores of 0.2 um or 0.45 um.

Crommelin et al, supra, further discusses the uses of 0.11 um or 0.4 um millipore filters to obtain sterilization of the final liposome product. It was determined that the loss of liposomes and their contents fluctuated between 0.3 and 18.6% for the same

liposome preparation. This variable loss was attributed to the pestle pressure which is applied manually and is thus never the same. Liposomes larger than 0.5 um cannot be sterilized by filtration as their integrity is destroyed and their contents set free when forced through 0.22 um pores. Consistent with the prior art, Crommelin et al stated:

> Other methods for sterilizing liposomes are hardly possible as all chemical or physical procedures would lead to the destruction of the integrity of their double membrane structure.

Thus, no motivation was provided for trying other types of sterlilization procedures.

Rao, L.S., "Liposomal Dosage Form Development - Some Practical Considerations", Journal of Parenteral Science and Technology, 37:73 (1987) further stated:

> In view of the difficulty of applying the normal methods of terminal sterilization such as membrane filtration and autoclaving to the final product, the processes chosen should be carried out under aseptic conditions. This demands filtration sterilization of the component solution and full validation of the procedures.

Another publication which follows in like vein is European Patent Application 0 211 647. This reference discloses mixtures of a hydrating agent such as arginine or glutamic acid and liposome-forming materials to provide a pre-liposome gel. This pre-liposomal gel is stated to be extraordinarily stable and thus stable enough to be autoclaved for sterilization. The pre-liposome gel spontaneously forms highly stable liposomes in aqueous solution. Consistent with the state of the art, there is no

suggestion to even consider subjecting the final liposome product to autoclave conditions.

The prior art has consistently maintained that liposomes would lose their integrity and degrade when subjected to heat sterilization conditions. This invention presents a method of non-destructively sterilizing or heating liposomes.

SUMMARY OF THE INVENTION

This invention includes a method for sterilizing liposomes comprising subjecting liposomes to heating at a sterilization effective temperature and for a sterilization effective time to achieve sterilization of the liposomes. On one embodiment, the liposomes are in aqueous liquid. In another embodiment, the liposomes contain a bioactive agent, such as an aminoglycoside, particularly gentamicin. Additionally included in the invention are multilamellar liposomes, particularly where the multilamellar liposomes are SPLV liposomes. In another embodiment, the liposomes comprise phospholipid, in particular egg phosphatidylcholine and cholesterol. In a further embodiment, the liposomes are from about 30 nm to about 2 microns in diameter. In a preferred embodiment, the liposomes are autoclaved using saturated or superheated steam which may be followed by slow cooling or quick cooling. In addition, the autoclave temperature may be from about 212 to about 300^{o}F, preferably about 220 to about 275^{o}F, and more preferably from about 250 to about 265^{o}F. Further included in the present invention is a method wherein the heating time is about ten to fifteen minutes once the liposomes reach the desired temperature. In another embodiment, a glass container, a butyl rubber stopper, an aluminum seal or any combination thereof is used on the container during heating. Another embodiment is a method of non-destructively heating liposomes comprising subjecting liposomes to heating at temperatures of about 212^{o}F or more, preferably about 212 to about 300^{o}F where the heating is more preferably conducted in an aqueous liquid.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Liposomes are completely closed lipid bilayer membranes containing an entrapped aqueous volume. In the present invention, the term lipid as used herein shall mean any suitable material resulting in a bilayer such that a hydrophobic portion of the lipid material orients toward the aqueous phase. Lipids further include highly hydrophobic compounds, e.g., triglycerides and sterols, e.g., cholesterol which can be incorporated into the bilayer. The lipids which can be used in the liposome formulations of the present invention are the phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylinositol (PI), sphingomyelin (SPM), and the like, alone or in combination. The phospholipids can be synthetic or derived from natural sources such as egg or soy. Particular synthetic phospholipids are dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG). The liposomes can also contain other steroid components such as polyethylene glycol derivatives of cholesterol (PEG-cholesterols), coprostanol, cholestanol, or cholestane, and combinations of PC and cholesterol. They may also contain organic acid derivatives of sterols such as cholesterol hemisuccinate (CHS), and the like. Organic acid derivatives of tocopherols may also be used as liposome-forming ingredients, such as alpha-tocopherol hemisuccinate (THS). Both CHS- and THS- containing liposomes and their tris salt forms may generally by prepared by any method known in the art for preparing liposomes containing these sterols. In particular, see the procedures of Janoff, et al., U.S. Patent No. 4,721,612 issued January 26, 1988, entiled "Steroidal Liposomes", Janoff, et al., PCT Publication No. WO 87/02219, published April 23, 1987, entitled "Alpha-Tocopherol Based Vesicles", filed September 24, 1986, respectively. The liposomes may also contain glycolipids.

Liposomes may be unilamellar vesicles (possessing a single bilayer membrane) or multilamellar vesicles (onion-like structures

characterized by multiple membrane bilayers, each separated from the next by an aqueous layer). The bilayer is composed of two lipid monolayers having a hydrophobic "tail" region and a hydrophilic "head" region. The structure of the membrane bilayer is such that the hydrophobic (nonpolar) "tails" of the lipid monolayers orient toward the center of the bilayer while the hydrophilic "head" orients towards the aqueous phase.

The original liposome preparation of Bangham, et al. (J. Mol. Biol., 1965, 12:238-252) involves suspending phospholipids in an organic solvent which is then evaporated to dryness leaving a phospholipid film on the reaction vessel. Next, an appropriate amount of aqueous phase is added, the mixture is allowed to "swell", and the resulting liposomes which consist of multilamellar vesicles (MLVs) are dispersed by mechanical means. This technique provides the basis for the development of the small sonicated unilamellar vesicles described by Papahadjopoulos et al. (Biochim. Biophys. Acta., 1968, 135:624-638), as well as large unilamellar vesicles.

Unilamellar vesicles may be produced using an extrusion apparatus by a method described in Cullis et al., PCT Application No. WO 87/00238, published January 16, 1986, entitled "Extrusion Technique for Producing Unilamellar Vesicles" incorporated herein by reference. Vesicles made by this technique, called LUVETS, are extruded under pressure through a membrane filter. Both small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) are suitable in the practice of the present invention.

Another class of liposomes are those characterized as having substantially equal intralamellar solute distribution. This class of liposomes is denominated as stable plurilamellar vesicles (SPLV) as defined in U.S. Patent No. 4,522,803 to Lenk, et al., monophasic vesicles as described in U.S. Patent No. 4,558,579 to Fountain, et al. and frozen and thawed multilamellar vesicles (FATMLV) wherein the vesicles are exposed to at least one freeze and thaw cycle; this procedure is described in Bally et al., PCT Publication No.

87/00043, January 15, 1987, entitled "Multilamellar Liposomes Having Improved Trapping Efficiencies" and incorporated herein by reference.

A variety of sterols and their water soluble derivatives have also been used to form liposomes; see specifically Janoff et al., U.S. Patent No. 4,721,612 issued January 26, 1988, entitled "Steroidal Liposomes". Mayhew et al., PCT Publication No. WO 85/00968, published March 14, 1985, described a method for reducing the toxicity of drugs by encapsulating them in liposomes comprising alpha-tocopherol and certain derivatives thereof. Also, a variety of tocopherols and their water soluble derivatives have been used to form liposomes, see Janoff et al., PCT Publication No. W087/02219, published April 23, 1987, entitled "Alpha Tocopherol-Base Vesicles".

During preparation of the liposomes, organic solvents may be used to suspend the lipids. Suitable organic solvents are those with a variety of polarities and dielectric properties, which solubilize the lipids, and include but are not limited to halogenated, aliphatic, cycloaliphatic, or aromatic-aliphatic hydrocarbons, such as benzene, chloroform, methylene chloride, or alcohols, such as methanol, ethanol, and solvent mixtures such as benzene:methanol (70:30). As a result, solutions (mixtures in which the lipids and other components are uniformly distributed throughout) containing the lipids are formed. Solvents are generally chosen on the basis of their biocompatability, low toxicity, and solubilization abilities.

Some liposomes may be dehydrated thereby enabling storage for extended periods of time until sue. Standards freeze-drying equipment or equivalent apparatus may be used to lyophilize the liposomes. Liposomes may also be dehydrated simply by placing them under reduced pressure and allowing the suspending solution to evaporate. Alternatively, the liposomes and their surrounding medium may be frozen in liquid nitrogen prior to dehydration. Such dehydration may be performed in the presence of one or more protectants such as protective sugars, according to the process of

Janoff, et al., PCT 86/01103, published February 27, 1986, and incorporated hereby by reference.

The present invention is not limited to the liposomes described above and any type of liposome may be autoclaved by the process of the present invention.

In a liposome-drug delivery system, a bioactive agent such as a drug may be entrapped in or associated with the liposome and then administered to the patient to be treated. For example, see Rahman, et al., U.S. Patent No. 3,993,754; Sears, U.S. Patent No. 4,145,410; Papahadjopoulos, et al., U.S. Patent No. 4,235,871; Schneider, U.S. Patent No. 4,114,179; Lenk, et al., U.S. Patent No. 4,522,803; and Fountain, et al., U.S. Patent No. 4,588,578. Pharmaceutical liposomal preparations are, in the preferred embodiment, delivered in physiological saline or water buffered with phosphate or citrate appropriate for injection.

All types of bioactive agents, both hydrophilic and lipophilic, when encapsulated in liposomes can be autoclaved by the process of the present invention. The only practical limitation in the bioactive agent selected is that it must be stable enough to withstand the autoclaving procedure. Obviously one would not select a bioactive agent which would decompose or degrade during the autoclaving process. As used in the present invention, the term bioactive agent is understood to include any compound having biological activity, e.g. drugs and other therapeutic agents such as peptides, hormones, toxins, enzymes, neurotransmitters, lipoproteins, glycoproteins, immunomodulators, immunoglobulins, polysaccharides, cell receptor binding molecules, nucleic acids, polynucleotides, and the like, as well as biological tracer substances such as dyes, radio-opaque agents, and fluorescent agents. The foregoing drugs are offered as guidance, and a wide variety of other suitable drug formulations are useful in the practice of the present invention. Such drug formulations may include aminoglycoside antibiotics, such as gentamicin, amikacin,

streptomycin and tobramycin and antineoplastic agents such as doxorubicin, vinblastine, vincristine, daunorubicin, mechlorethane hydrochloride, mitoxanthrone, cyclophosphamide, mitomycin, blenycin and cisplatin. For additional types of drugs, reference may be made to any standard medical or pharmaceutical reference text, e.g., Goodman and Gilman, "The Pharmacological Basis of Therapeutics", which is herein incorporated by reference.

In addition, the liposomes may contain in vitro diagnostics, e.g., an assay for antibodies, bacteria, dyes and the like.

The drug-containing liposomes of this invention may be administered to any plant or animal including humans.

Administration will also be understood to include extracorporeal use such as directed to blood or serum collected from an animal.

A therapeutically effective amount of any of the foregoing bioactive agents will be understood to mean a sufficient amount to achieve a desired physical or physiological response, and for known drugs will generally be the same dose for the existing dosage forms of the drug.

The therapeutically effective amount of a given pharmacological agent will vary with the purpose of the administration, the particularities of the recipient and other facts well known in the art.

For administration to humans in the curative treatment of disease states, the prescribing physician will ultimately determine the appropriate dosage for a give human subject, and this can be expected to vary according to the age, weight, and response of the individual as well as the nature and severity of the patient's disease. The dosage of the drug in liposomal form will generally be about that employed for the free drug. In some cases, however, it may be necessary to administer doses outside these limits.

The mode of administration of the preparation may determine the sites and cells in the organism to which the compound will be delivered. The liposomes of the present invention can be administered alone but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. The preparations may be injected parenterally, for example, intra-arterially or intravenously. The preparations may also be administered via oral, subcutaneous, or intramuscular routes. For parenteral administration, they can be used, for example, in the form of a sterile aqueous solution which may contain other solutes, for example, enough salts or glucose to make the solution isotonic. Other uses, depending upon the particular properties of the preparation, may be envisioned by those skilled in the art.

The pharmacologically active compounds of this invention can be processed in accordance with conventional methods of galenic pharmacy to produce medicinal agents for administration to patients, e.g., mammals including humans.

The compounds of this invention can be employed in admixture with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral (e.g., oral) or topical application which do not deleteriously react with the active compounds. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatins and carbohydrates, e.g., lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxy methylcellulose, polyvinyl pyrrolidone, and the like. The pharmaceutical preparations can be sterilized, and if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds. They

can also be combined where desired with other active agents, e.g., vitamins.

For parenteral application, e.g., intramuscular, intraperitoneal, intravenous or subcutaneous, particularly suitable are injectable, sterile solutions, preferably oily or aqueous solutions, as well as suspensions, emulsions, or implants, including suppositories. Ampoules are convenient unit dosages.

For enteral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules. A syrup, elixir, or the like can be used and a sweetened vehicle may be employed.

Sustained or directed release compositions can be formulated, e.g., liposomes or those wherein the active compound is protected with differentially degradable coatings e.g., by microencapsulation, multiple coatings, and the like. It is also possible to freeze-dry the new compounds and use the lyophilizates obtained, for example, for the preparation of products for injection.

For topical application, there are employed as nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, aerosols, and the like, which are, if desired, sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. For topical application, sprayable aerosol preparations are also suitable wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., a freon.

The method of non-destructively heating of liposomes preferably is employed with liposomes in aqueous liquid such as pharmaceutically acceptable carriers (e.g., water or saline). "Non-destructively" shall mean that the liposome bilayer structure is present after the heating.

Obviously, the liposomes may be any size in order to be heated by the procedure of the present invention. Preferably, the liposomes will be in an aqueous liquid carrier such as water or saline and including gels. Typically, the liposomes are from about 30 nm to about 2 microns in diameter, and the liposome may contain from about 50% to about 100% of a bioactive agent.

There are also a wide variety of types of heating apparatus for conducting the method of the present invention. Such apparatus are described, for instance, in Remington's Pharmaceutical Sciences, Fifteenth Edition (1975), which is herein incorporated by reference. One type of heating apparatus is the autoclave. A particular autoclave is a gravity or downward displacement autoclave which depends on the difference in density between air and steam. Air, being heavier, is displaced to the bottom of the chamber and exists while the steam is admitted near the top of the chamber. In such units, the temperature at the drain point is usually measured to assure that no more air is moving out of the system. Modern-day hospital autoclave modles are available in a wide range of sizes, from about 12 inches in diameter to 6 feet or larger in diameter.

Any method of heating may be used in the process of the present invention. Such methods include using saturated or superheated steam, using high pressure water, ethylene glycol or the like at elevated temperatures (usually greater than 250°F) in an immersion bath, using electromagnetic radiation of suitable wavelength, using a shaking mechanism in the autoclave in conjunction with any of the foregoing methods or using a quick cooling or slow cooling procedure following any of the above-mentioned autoclave procedures. With respect to this last point, some autoclaves have outside jackets

which are equipped with a cold water cycle that will rapidly cool the load to facilitate removal. A liposomal product so autoclaved will conform to the sterility test such as those incorporated in US Pharmacopeia (USP) XXI, British Pharmacopeia and the European Pharmaceutical Register (Ph. Eur.)

"Slow cooling" will be understood to mean cooling a heated preparation by unassisted dissipation of heat from a heating apparatus. "Quick cooling" shall be understood to mean reducing the temperature of the heated preparation to a below ambient temperature (generally about 20 to 25%) by direct contact cooling (e.g., cold water or cold air) generally within about 10 minutes.

The temperature used during the autoclaving process must be a temperature which is "sterilization effective", i.e., that which will achieve the necessary sterilization. In addition, it is necessary to consider how long it will take for the liposomes to reach the correct temperature and how long after reaching the required temperature they should be held to achieve sterility. A heating time which is sufficient to achieve sterility will be a sterilization effective time. Generally, the greater the volume of material to be autoclaved, the longer the time necessary to achieve sterilization. For instance, for small volumes (up to 250 ml), the time required to reach thermal equilibrium is short and the standard fifteen minutes will usually suffice. Larger, volumes will often require a longer heating period before all the liposomes have reached the desired temperature. As long as about one hour or longer at 121°C may be required to sterilize liposomes in an 8 liter standard pyrex bottle. The USP (United States Pharmacopeia) defines steam sterilization as heating in an autoclave employing steam-saturated air under pressure for at least fifteen minutes at a minimum temperature of 121°C. The measurement of time begins when the temperature of the material being sterilized reaches 121°C.

Frequently, the temperature selected to autoclave the liposomes is from about 212 to about 300°F or more (about 100 to about

150°C), preferably from about 220 to about 275°F (about 104 to about 136°C), and more preferably the temperature is from about 250 to about 265°F (about 121 to about 130°C). The duration of the autoclaving procedure as noted above is dependent both on the temperature selected as well as the volume of material to be autoclaved. Preferably, those conditions are selected such that the duration of the autoclaving procedure once the container reaches the desired temperature is from about ten to fifteen minutes. The methods used to autoclave should conform to Good Manufacturing Practice (GMP) as defined by the FDA.

Any sterilizable container may be used to hold the liposomes for autoclaving. Such a container must, of course, be able to withstand the autoclaving process, e.g., be impervious to high temperatures. Glass is the most common type of material used to retain liquids for autoclaving since it is able to withstand autoclaving without resulting in physical distortion. Glass is also a relatively unreactive and inert substance. Additionally, it provides excellent protection against water vapor and gas permeation. Two definite disadvantages of glass in the field of packaging, however, are its fragility and weight. A variety for other materials including plastic packaging materials may be suitable. The use of plastics in the health care industry has grown at a very rapid rate. This phenomenal growth is primarily due to the wide flexibility in choice of properties offered by plastics. Suitable plastics are discussed in Remington's Pharmaceutical Sciences, Fifteenth Edition (1975), herein incorporated by reference. Suitable types of sterilizable containers are well known in the art and include but are not limited to syringes, bottles, vials, ampoules and bags.

Any suitable closure able to withstand the autoclaving process may be used. Typical closure materials are well known in the art and include plastics (polymers), rubbers, metals, glass and the like. The seal is preferably made of metal and is strong enough to retain a tight seal around the mouth of the container during autoclaving.

The following examples are given for purposes of illustration only and not by way of limitation on the scope of the invention.

EXAMPLE 1

INTEGRITY OF HEATED LIPOSOMES

Gentamicin, an aminoglycoside antibiotic, was encapsulated in egg phosphatidylcholine (EPC) according to a modified stable plurilamellar vesicle process (SPLV) as disclosed in U.S. Serial No. 946,398, filed December 23, 1986. This was done in order to evaluate the efficacy of terminal sterilization by autoclaving.

60 ml glass vials filled with 50 ml of liposomes containing gentamicin in saline were autoclaved. The vials were stoppered with butyl rubber and sealed using aluminum seals. The autoclaving experiments were conducted at a temperature of about 250°F. The duration of the autoclaving process varied between about ten to fifteen minutes.

After autoclaving, the vials were either quick cooled (in a ice bath at approximately 4° to 6°C for 15 minutes) or slow cooled (left at room temperature for 1 hour). Liposomes that were not autoclaved served as controls. Both the control vials and autoclaved vials are identified in Table I.

In Experiments 1 and 2, the 60 minute slow cool time was done at room temperature. In Experiments 3 and 4, the 15 minute quick cool was conducted at approximately 4 to 6°C in an ice bath. The cooling in Experiments 5 and 6 involved a water spray to decrease the temperature to 40°C in approximately 10 minutes.

After autoclaving, the content of both the autoclave vials and controls were evaluated for lipid integrity and leakage of the drug. Lipid integrity tests were conducted using thin layer chromatography (TLC) by spotting 100 ug of sample on an Analtech HPTLC-HL pre-adsorbent plate. The plate was developed in a mobile

phase of chloroform (60) : Methanol (35) : Ammonium Hydroxide (5). Visualization was accomplished by dipping the plate into $CuSO_4$ solution (10%) and heating to 180°C. This method allowed detection of lysophosphatidyl choline (LysoPC) and free fatty acids (FFA) which are all visible degradation products of EPC.

Drug leakage was examined by the aminoglycoside spectrophotometric assay employing Picrylsulfonic acid as the reacting agent to determine gentimicin concentration. The results are set forth in Table II below:

The last vial number for each Experiment (either c or e) represented the control.

As noted above, autoclaving the sample for 10 to 15 minutes at 121°C or 250°C does not affect the liposome product to any significant extent with respect to its ability to retain entrapped drug or lipid integrity. The drug leakage overall was about 5%. The quick cool vials averaged about 6%, and the slow cool samples averaged about 3%. When autoclaved for periods of about 30 to 60 minutes, leakage climbed to 26-28%.

The case is the same for lipid integrity. Lyso PC content averaged 0.5 to _5% for 30 to 60 minute autoclaved samples. FFA content was minimal (2 to 3%, except for vial no. 5d) in samples autoclaved for 10 to 15 minutes, but reached 5 to 15% in those autoclaved for longer periods.

These experiments show that when autoclaved (preferably for 10 to 15 minutes) between 121°C and 250°C, the liposomes are not destroyed, do not degrade, and their contents do not leak out to any appreciable extent. In addition, a slow cool down seems preferable.

EXAMPLE 2

In order to test the efficacy of heating terminally sterilizing liposomal products, liposomes were seeded with Bacillus stearothermophilus. No antibiotics were used in this procedure. These liposomes containing the Bacillus were placed in a 50 ml glass container with a butyl rubber stopper, sealed with aluminum seals and autoclaved at about 250°F for about fifteen minutes. The contents of the glass container were cooled and plated to test the presence of bacteria. It was determined that the bacteria had been completely eliminated by the autoclaving. Thus, sterilization was achieved. This example demonstrated that the autoclaving method of the present invention is useful in sterilizing both "empty" liposomes as well as those containing drugs.

It is to be understood that the invention is not limited to the specific examples described hereinabove which are given only by way of illustration, and that modification may be made within the scope of the appended claims without departing from the spirit of the invention.

TABLE I

Summary of Results

Experiment	Total No. of Samples	No. of Samples Autoclaved	No. of Control Samples	Cooling Time (minutes)	Autoclave Temp. (°C)	Autoclave Time (minutes)
1	3	2	1	60	250	10
2	3	2	1	60	250	15
3	3	2	1	15	250	10
4	3	2	1	15	250	15
5	5	4	1	10	121	10
6	5	4	1	10	250	30-60

Table II

Summary of Lipid Degradation and Drug Leakage

Experiment	Vial No.	Autoclave Temp. (°C)	Autoclave Time (min)	Cooling Time (min)	LysoPC (%)	FFA (%)	Drug Leakage (%)
1	1a	250	15	60	≤ 3	≤ 2	4.81
	1b	250	15	60	≤ 3	≤ 2	5.02
	1c	–	–	–	≤ 1	0	0.67
2	2a	250	10	60	≤ 3	≤ 2	1.61
	2b	250	10	60	≤ 3	≤ 2	1.77
	2c	–	–	–	≤ 3	0	0.00
3	3a	250	10	15	≤ 3	≤ 2	6.68
	3b	250	10	15	≤ 3	≤ 2	5.44
	3c	–	–	–	≤ 2	0	1.00
4	4a	250	15	15	≤ 5	≤ 2	7.83
	4b	250	15	15	≤ 5	≤ 2	8.11
	4c	–	–	–	≤ 4	0	3.41
5	5a	121	10	10	≤ 3	≤ 3	8.63
	5b	121	10	10	≤ 2	≤ 2	6.54
	5c	121	10	10	≤ 2	≤ 2	9.75
	5d	121	10	10	≤ 3	≤10	8.25
	5e	–	–	–	≤ 0.5	0	1.26
6	6a	250	30	10	≤15	≤5-7	28.07
	6b	250	30	10	≤15	≤5-7	26.55
	6c	250	60	10	≤10	≤15	26.96
	6d	250	60	10	≤10	≤15	26.27
	6e	–	–	–	≤ 2	≤ 1	1.67

What is claimed is:

1. A method for sterilizing liposomes comprising subjecting liposomes to heating at a sterilization effective temperature and for a sterilization effective time to achieve sterilization of said liposomes.

2. The method of claim 1 wherein the liposomes are in aqueous liquid.

3. The method of claim 2 wherein said liposomes contain a bioactive agent.

4. The method of claim 3 wherein said bioactive agent is an aminoglycoside.

5. The method of claim 4 wherein said aminoglycoside is gentamicin.

6. The method of claim 2 wherein said liposomes are multilamellar.

7. The method of claim 6 wherein said multilamellar liposomes are SPLV liposomes.

8. The method of claim 2 wherein said liposomes comprise phospholipid.

9. The method of claim 8 wherein said liposomes comprise egg phosphatidylcholine and cholesterol.

10. The method of claim 2 wherein the liposomes are from about 30 nm to about 2 microns in diameter.

11. The method of claim 1 wherein the heating is autoclaving.

12. The method of claim 11 wherein the autoclaving is conducted using saturated or superheated steam.

13. The method of claim 12 wherein the autoclaving procedure is followed by a slow cooling procedure.

14. The method of claim 12 wherein the autoclaving procedure is followed by a quick cooling procedure.

15. The method of claim 11 wherein the autoclave temperature is from about 212 to about 300°F.

16. The method of claim 15 wherein the autoclave temperature is from about 220 to about 275°F.

17. The method of claim 16 wherein the autoclave temperature is from about 250 to about 265°F.

18. The method of claim 2 wherein the heating time is about ten to about fifteen minutes once said liposomes reach the desired temperature.

19. The method of claim 2 wherein said liposomes in aqueous liquid are in a glass container during autoclaving.

20. The method of claim 2 wherein said liposomes in aqueous liquid are in a container comprising a butyl rubber stopper.

21. The method of claim 2 wherein said liposomes in aqueous liquid are in a container comprising an aluminum seal.

22. A method of non-destructively heating liposomes comprising subjecting liposomes to heating at temperatures of about 212°F or more.

23. A method of non-destructively heating liposomes comprising subjecting liposomes to heating to a temperature of about 212 to about 300°F.

24. The method of claim 22 wherein liposomes are in an aqueous liquid.

25. The method of claim 23 wherein the liposomes are in an aqueous liquid.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US89/04354

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4):A61L 02/00
US Cl.: 422/01

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	422/1,21,25,26; 424/450; 428/402.2; 436/829

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [6]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US,A, 4,614,796 (KAWAMATA ET AL.) 30 September 1986 See entire document.	1-3,6,8,9,11,12,15,16,19,22-25 13,14,17,18,20,21
Y	US, A, 4,522,803 (Lenk et al.) 11 June 1985 See entire document.	8- 10
X,P Y	US, A, 4,839,142 (CHARM) 13 June 1989 See entire document.	1,2,22-25 11-21

[6] Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [1]	Date of Mailing of this International Search Report [1]
29 DECEMBER 1989	16 JAN 1990
International Searching Authority [1]	**Signature of Authorized Officer [19]**
ISA/US	WILLIAM H. BEISNER

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